Crossing Brachypodium

Beta version

John Vogel, May 5, 2009

Comments

I have tried this method a few times with good success. I have not done a lot of cross yet so don't have a really good feel for all the variables and how successful it will be in general. However, I thought you would like to see this sooner than later in case you are setting up crosses now. Please let me know if you try this and how it works for you. I will be setting up a lot more crosses as soon as I have plants at the proper stage in few weeks.

This method is based on the fact that immature anthers will dehisce shortly after removal from the plant. This provides a lot of fresh pollen for crossing. I use a dissecting microscope so it is very obvious what stage everything is at and if you successfully pollinated the flowers. I can make a cross in about 5-10 minutes and this will probably get faster with practice.

Materials

Dissecting microscope
fine tweezers (Ted Pella Inc. Cat# 5622)
microscope slides
small scissors (Spring-type micro scissors work great for this. Ted Pella Inc. Cat # 1346)
(optional) Stand-mounted or visor-type magnifying glasses. These are useful for picking out flowers at the right stage.

Growth conditions and timing

I have successfully used plants grown in a growth chamber with 20 hr days and a greenhouse at 16 hour days. It is very important to have plants at the proper stage for crossing. I have been using the first few large tillers that are produced by a plant. Each plant will only be at the proper stage for a few days. For a pot of 10 plants I think there may be flowers at the proper stage for about a week. After that, you could try to use some of the later secondary tillers, but I haven't tried that yet. When crossing different ecotypes it is probably a good idea to make several plantings separated by a few days to make sure you have plants at the right stage at the same time. I don't know if the time of day is important, but have noticed that a greater percentage of the anthers picked in the afternoon dehisced than those picked in the morning. However, the sample size here is very small so I need to look at this more closely.

Fig. 1. Preparing pollen
Select flowers with very mature anthers. You can bend back the lemma with your hand to see the anthers. Remove the palea and flower by pulling the palea away from the lemma. (A and B) Proper stage flower. (C) same flower with top side of palea removed for a better view of the anthers. Note it is not necessary to remove the palea when removing anthers. (D) Ripe anthers on a microscope slide. (E) Same anther 25 minutes later. Note that it has dehisced and shed over a hundred pollen grains. I typically pick out 14 anthers and after 20-40 min some usually dehisce. The percentage varies from 0 to 50% and I think more dehisce in the afternoon than morning, but need to do more replication to be sure. (A-E) Scale bar is 1 mm.

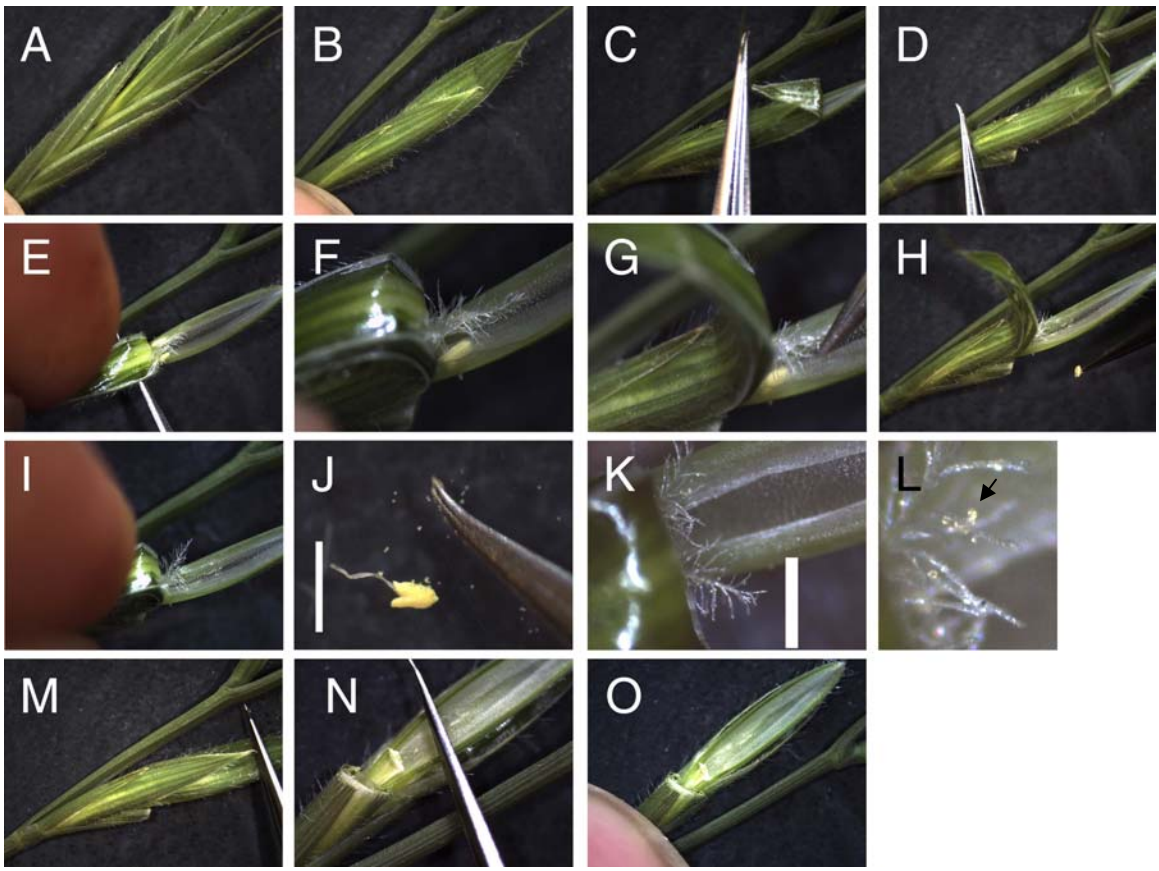

Figure 2. Prepare the female and pollination.
To identify flowers at the proper stage, pull the lemma back and look at the flower. You want flowers that are as mature as possible, but before the anthers dehisce. A stand-mounted magnifying glass or visor is useful for this. (A) Proper stage inflorescence. (B) Remove all flowers except the one target for crossing. I use either the first or second flower on the inflorescence. (C) Fold back lemma. (D) To fold back lemma without damaging the flower you can place your tweezers across the lemma where shown then pull back lemma with fingers to expose the flower as shown in (E). (F) Flower ready for emasculation. (G) Remove anthers by inserting forces in fold of palea and pulling out anther. Take care not to puncture or damage palea. Under our conditions the palea is necessary for normal seed development. (H) Anther comes out easily. Examine carefully to make sure no pollen has fallen out. Repeat on other side. (I) Emasculated flower. (J) Pick up pollen with closed tweezers. Usually several pollen grains will adhere to tweezers. You might also use a few fine brush bristles. Apply pollen to stigma by very carefully brushing tweezers across the stigma. (K-L) Stigma with pollen grains. (M) Carefully close palea over lemma. (N) Turn flower over and carefully push palea back into place. (O) Final pollinated flower.

Good luck!